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Cultivation of psorospermia (a preliminary communication).

By Sheridan Delépine, M.B.Edin.

[With Plate VII, figs. 1., and 2; and Plate VIII.]

Bodies which resemble closely several forms of gregarinæ or psorospermia have been described during the last fifty years by numerous observers as occurring in various organs in man. I need only refer by name to those observers to show that, notwithstanding the comparative rarity of their observation, they must be received as indicating the possibility of the infection of man by these parasites. They have been seen or described by *Gubler* (1858). *Virchow, Dressler, Sömmering, Sattler, Silcock,* in the liver; *Kjellberg, Eimer, Rivolta, Grassi,* in the intestine and fæces during life; *Lindemann, Knoch* on the hair and in several internal organs; *Künstler* and *Pitres* in the pleura; *Hadden, Cobbold, Eve,* in the kidney and ureters; *Robin, Lebert, Lindemann, Bollinger, Darier, Wickham, Hutchinson, jun., Bowlby,* in the skin, specially in molluscum fibrosum and Paget's disease of the nipple. Even admitting a certain number of erroneous statements, in presence of such a series of observations there can be little doubt as to the occasional presence of psorospermia in human tissues. It would, therefore, be unwise to reject without inquiry the suggestion which has been made by several observers that psorospermia had an important share in the production of certain lesions, such as Paget's disease of the nipple or epitheliomata. I must own that for years I, like many others, have been struck with the appearance of certain large bodies occupying or not the central parts of cell nests, and often present in epitheliomata. I had generally considered those

large round or oval bodies as the results of some degeneration of the cells; but when the possibility of the presence of psorospermia in epithelial tumours was suggested, it seemed to me that this hypothesis might possibly be correct. I have for the last two years observed a large number of epitheliomatous tumours of external and internal organs, in which such bodies were present, and have found that, although there was a remarkable resemblance between them and undoubted psorospermia, they entirely failed to respond to any of the chemical tests which I have found to give definite reactions with certain forms observed in the rabbit's liver. I have also failed to find any of the developmental forms which can so easily be seen side by side in the rabbit's liver and intestine. The tumours in which I have noticed the presence of the psorospermoid bodies were—1. Several epitheliomata of the lips and tongue. 2. Two epitheliomata of the œsophagus. 3. A primary carcinoma of the bile-ducts and liver. 4. Epitheliomata of the skin. 5. An epithelioma of the vagina. 6. Two epitheliomata of the vulva.[1]

In all these cases I have seen forms which seemed entirely similar to some varieties of psorospermia; yet, for the reasons I have already given, in none did I feel justified in recording them as evidences of the existence of psorospermia in epithelial growths. On the other hand, I had observed absolutely similar lesions in parts where the presence of psorospermia seemed entirely out of the question, and the existence of degenerative processes was equally evident.

In presence of such a state of things I thought that it would be desirable to find whether one could easily, by cultivation, obtain changes which might be used partly for diagnostic and partly for inoculation purposes. Kauffmann had already, in 1847, succeeded in cultivating psorosperms in water, or rather he had observed that the contents of the capsules divided into four spores when placed in water. On repeating this experiment I was surprised to find that this phenomenon was observable only in a small proportion of the parasites obtained directly from the liver of a rabbit. In fact, when pure water was used the changes consisted only in a swelling of the capsule, which became gradually very transparent and almost invisible, whilst the protoplasmic contents assumed the appearance

[1] See communication made at the International Congress of Hygiene and Demography, 'Brit. Med. Journ.,' 1891, ii, p. 362.

of a large, very granular amœboid mass, with sometimes an indistinct nucleus. Such masses seemed to become more and more coarsely granular or vacuolated, until they broke down into clumps of granular *débris*. The few oviform bodies in which subdivision of the contents occurred, according to the description given by Kauffmann, did not seem to undergo any further change.

As the psorosperms in which these indications of division occurred were in the midst of other organisms which were breaking down, I suspected that those in which evidences of division had occurred were living on the substance of their decaying neighbours.

This first experiment indicated clearly that water is not a very suitable material for cultivating psorosperms.

I then attempted to cultivate psorosperms in interlamellar films,[1] so as to be able to follow the changes occurring in individual organisms kept in various media. Psorosperms taken from a single psorospermic nodule (which had become cystic by accumulation of serum in the lumen of the diseased and obstructed bile-duct) were placed in bile, in exuded serum, in glycerine, and nutrient gelatine, and kept at a temperature of about 20° Centigrade. In less than a week the majority of the psorosperms placed in serum showed complete or partial subdivision of their protoplasma into four or more round or oval bodies, similar to those described by Kauffmann.[2] Many of those kept on nutrient gelatine showed the same changes, but the process was not quite so advanced, nor did it affect quite so large a number of psorosperms as in the serum cultivations. The parasites kept in bile did not seem either to decay or to show any tendency to multiplication. The protoplasmic contents which, on removal from the liver, occupied, in most cases, the whole capsule, generally contracted rapidly so as to form, in the middle or at one end of the capsule, an oval or spherical body, generally full of highly refracting granules of rather large size. In fact, they had taken the form which attracted specially Hake's attention.

In the serous fluid taken from the nodule which had been selected for the purposes of inoculation there were, in addition to the ordinary encapsulated form, numerous naked parasites. These varied much in size, but were all smaller than the encapsulated form. They looked like gelatinous and homogeneous masses of

[1] See 'Lancet,' June, 1891.

[2] Note the absence of toxic properties of the serum in this case.

protoplasm, had a kind of yellowish colour, and some were evidently endowed with amœboid motion.

The same body was also found in the stomach and intestine, but in these organs the smaller varieties were predominant; whilst in the bile-ducts all the stages between the small, hardly defined jelly-like mass, and the large oval bodies with or without a nucleus or large granules, could also be found abundantly. The largest naked forms were of about the same size as the smallest encapsulated ones.

These naked forms kept well for weeks in bile, but I was not able to satisfy myself that any had become encapsulated. This may, however, come from my having been unfortunate in the selection of the individuals I was specially watching day by day; for, looking at the cultivations as a whole, I am under the impression that the total number of encapsulated psorosperms had augmented before the end of the month.

The same phenomenon seemed to occur in serum, but here again I was not able to watch the actual passage from the naked into the encapsulated form.

In the nutrient gelatine I soon lost sight of the naked gregarinæ.

The changes occurring in the encapsulated oviform bodies kept in serum, glycerine, and nutrient gelatine were mainly the following:

1. The contents contracted, sometimes showing distinct alterations of shape.

2. The granules or some internal network became in some arranged equatorially, so as to leave two clear poles; this was, however, distinct only in a few cases.

3. The protoplasmic mass divided into two oval or irregular bodies, capable of amœboid motion.

4. Then each of these subdivided into two again, and as this did not always occur at the same time, there were often three oval or round or irregular protoplasmic bodies free within the capsule. The capsule itself became larger, probably owing to accumulation of fluid within its cavity. At this stage one part of the protoplasma of the daughter-cells was often quite homogeneous and transparent, while the other part was finely granular. Small round bodies, strongly refractive, were generally visible in the granular part of the protoplasm.

5. When four daughter-cells or spores were present within the

mother-cell or cyst, they generally assumed for a short time a regular oval form, and they looked like diminutive oviform psorosperms. They seemed to have a thin capsule, but I have not been able to see it. They were almost entirely composed of a clear translucent elongated body folded upon itself, and often containing the round refractive bodies alluded to above. In some cases they seemed really to contain a folded or hooked body, resembling to a certain extent the *falciform corpuscles* of Eimer, Schneider, and other observers; *but this was only a transient appearance.*

6. These four bodies sometimes subdivided still further, so that the original capsule contained five, six, and even more small rounded bodies, which generally had a bright globule in their midst, and looked very much like small lymph-corpuscles.

7. The next change was, however, rendered manifest by a great alteration in shape, the four or more daughter-corpuscles becoming elongated and exhibiting slow amœboid motion, taking in turn vermiform, pyriform, and round shapes, and moving from one place of the capsule into the other. Whilst these changes were taking place the vermiform bodies occasionally became twisted together or seemed to coalesce, so that at times the cyst looked as if it contained only one thick spiral or wavy filament. There were in these amœboid bodies generally two of the bright bodies alluded to above.

8. At the same time bodies similar to these small bright round bodies became abundant in the nutrient material surrounding the psorosperms. They were at first smaller than red blood-corpuscles. They were often attached to the capsule, as if just passing through it; it was not very uncommon to find one at each pole of the capsule. I have, however, been unable to watch their actual escape.

9. At the end of three weeks many of the small bright corpuscles which had thus apparently become free showed distinct proof of multiplication, being often in pairs as shown in the plate. These were of the same size as, and resembled closely, some of the small bodies found in the stomach and intestine.

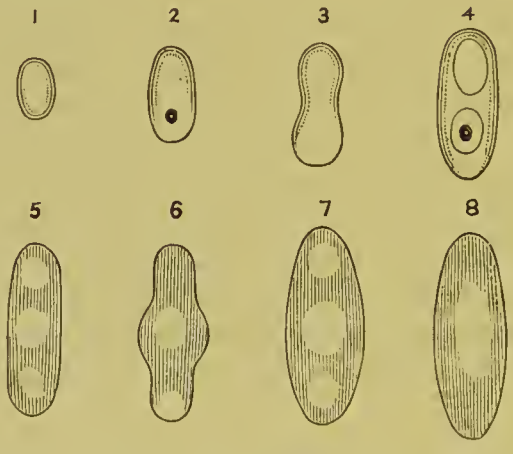
One of the most remarkable features of this process of reproduction, as observed under artificial circumstances, is the apparent possibility of the various stages described occurring indifferently after division of the mother-cell into two, three, four, five, six, and perhaps more daughter-cells.

By drawing the small bodies at intervals with the camera lucida I have been able to satisfy myself of their alteration of shape and of their movements from one place to another, so that I am certain that they are not the result of a degeneration process and of a breaking down of the protoplasma.

The accompanying sketches (Fig. 1, and Pl. VIII), all drawn to the same scale and copied from accurate drawings taken with the camera lucida, show the most important forms which I have observed and alluded to above.

I have so far failed to discover anything like any of these stages in the tumours I have examined. I feel, therefore, very sceptical as to the nature of the psorospermial bodies I have seen in them, and which are quite similar to those observed in Paget's disease of the nipple, as I have been able to ascertain by the examination of the specimens of Mr. Bowlby and Mr. Hutchinson, jun.

Fig. 1.



Young psorospermia found in the stomach of rabbits. 1, 2, 3, 4, 5, 6. Forms which seem to lead to 7, 8, but the nature of which is only surmised by analogy. 7, 8. Typical young psorosperms.

I think, however, it would be injudicious to speak positively before numerous attempts have been made to cultivate the suspicious cells found so abundantly in Paget's disease of the nipple and some epithelial tumours. By publishing a simple method capable of yielding satisfactory results in the case of true psoro-

spermia, I hope to encourage others to try cultivation experiments in all cases where the presence of psorospermia is suspected; and I trust that in this way a sufficient amount of information may be obtained to allow of a definitive settlement of this question. *May* 23*rd*, 1891.

Note.—I have purposely avoided to allude here to the meaning of the changes I have described, and to their bearings upon the modes of propagation and infection. I would refer those who take an interest in the question to my previous paper in the Pathological 'Transactions,' where the opinions of the most competent observers are discussed, with some bibliographical references.

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DESCRIPTION OF PLATE VII.

Figs. 1 and 2 illustrating Dr. Delépine's paper on Psorospermia.

Fig. 1.—Section through the walls of the psorospermial nodule which was used in the cultivation experiments described in the paper.

Fig. 2.—The same, less highly magnified, to show the papilliferous nature of the growth.

Both specimens were photographed by Mr. Andrew Pringle from specimens prepared by the author. In Fig. 1 the psorospermia appear as dark encapsulated bodies in the midst of the paler epithelium.

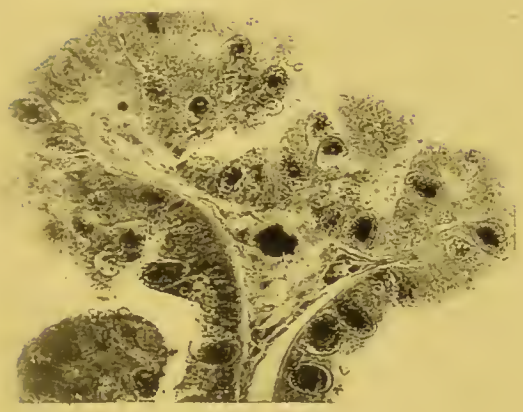


Fig. 1.

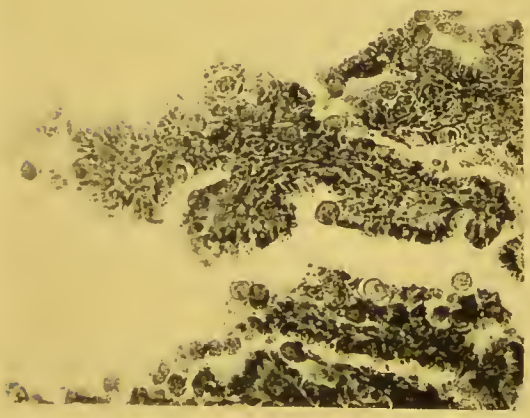


Fig. 2.

Danielsson & Co., Photo. Print.

DESCRIPTION OF PLATE VIII.

To illustrate Dr. Delépine's paper on the Development of Psorospermia.

(All the figures were drawn by the author with the camera lucida from fresh specimens obtained from the stomach, duodenum, large intestine, gall-bladder, bile-ducts, or cultivations; they have all been reduced to the same scale = × 1000. The first eight figures, representing chiefly the forms found in the stomach, are in the text.)

FIGS. 9—14.—Forms found in the duodenum, bile-ducts, and gall-bladder (and also in other parts).

FIGS. 9—11.—Naked forms, apparently motionless, found chiefly in bile.

FIG. 12, 13.—Very common forms, both in the intestine and in the liver.

FIG. 14.—Form generally observed after the psorosperms have been allowed to stand on the slide, probably the result of contraction of the protoplasm.

FIGS. 15—19.—Forms observed in cultivations during the first few days (within the first week).

Fig. 15.—Protoplasm contracted within the capsule and showing differentiated parts, the arrangements of which suggests karyokinesis.

FIGS. 16, 17, 19.—Division of the protoplasmic contents within the capsule, each amœboid mass thus produced is endowed with amœboid motion.

FIG. 18.—Appearance observed several times, and possibly indicating some kind of conjugation.

FIGS. 20—29.—Forms observed in cultivations during the second week. (Many of these were already visible during the first week, and in many cases did not alter rapidly after the second week.)

FIG. 20.—Young amœboid germs in a state of active amœboid motion.

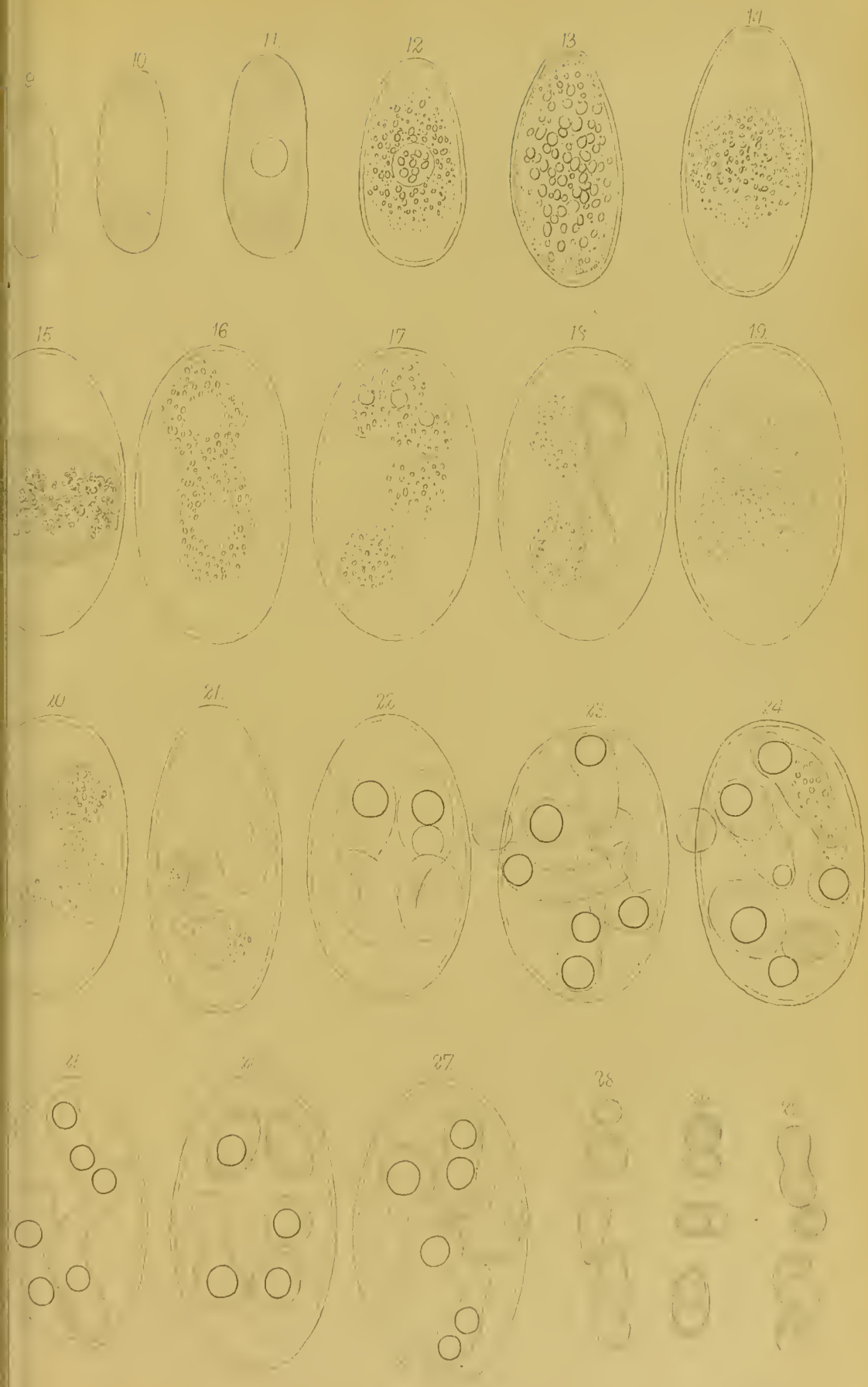
FIGS. 21, 22.—Capsules containing young psorspermin or pseudo-navicellæ, in some of which the *falciform* or *sickle-shaped* body is distinct. Of all the stages this is the one which remains evident longest.

FIGS. 23—26.—Further changes occurring in cultivations within the capsules, but which probably, under ordinary circumstances, would have occurred after liberation of the germs.

FIG. 27.—Germs showing distinct amœboid motion. Forms observed in cultivation during the second, third, and fourth weeks.

FIG. 28.—A few of the forms observed early.

FIG. 29.—Forms found abundantly by the end of the first month in the nutrient material about the capsules. The passage of these bodies through the capsules was not observed.


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